

CLAIMS

1. A polypeptide which is a synthase or transferase obtainable from a bacterium of the family *Mycobacteriaceae*, such as of the genus *Propionibacterium*.
- 5 2. A polypeptide according to claim 1 which:
 - (a) acts as an amide synthase or a phospho-, nucleotidyl- or aryl transferase; or
 - (b) has an activity within EC 6.3.1-, EC 2.7.7-, EC 2.7.8- or EC 2.5.1.17; and/or
 - 10 (c) is obtainable from a microorganism of the Sub order *Propionibacterineae* or *Propionibacteria freudenreichii*.
3. A synthase polypeptide according to claim 1 or 2 comprising:
 - (i) the amino acid sequence of SEQ ID No. 2, 4, 6 or 8; or
 - (ii) a variant of (i) which is a synthase or transferase; or
 - 15 (iii) a fragment of (i) or (ii) which is a synthase or transferase.
4. A polypeptide according to claim 1 wherein the variant in (ii) has at least 70%, 75%, 80% or 85% identity to the amino acid sequence of SEQ ID No. 2, 4, 6 or 8 (e.g. at least 85% identity to SEQ ID No.8) and/or the fragment of (iii) is at least 150 amino acids in length.
- 20 5. A polypeptide according to any preceding claim which is obtainable from a Gram positive bacterium and/or is a cobyrinic acid -a,c-diamide synthase, a cobinamide kinase, a cobinamide phosphate guanyltransferase, a cobalamin (5'-phosphate) synthase or an adenosyl transferase.
6. A polynucleotide comprising:
 - 25 (a) the nucleic acid sequence of SEQ ID No. 1, 3, 5 or 7 or a sequence encoding a polypeptide according to any preceding claim;
 - (b) a sequence which is complementary to, or which hybridises to, a sequence as defined in (a);
 - (c) a fragment of a sequence in (a) or (b);
 - 30 (d) a sequence having at least 60% identity to a sequence as defined in (a), (b) or (c); or

- (e) a sequence that is degenerate as a result of the genetic code to any one of the sequences as defined in (a) to (d).

7. A sequence according to claim 7 wherein in (b) the hybridisation is under stringent conditions, the fragment in (c) is at least 20 bases in length (such as at least 510 bases for a fragment of SEQ ID No. 7) and/or the identity in (d) is at least 70% or 80% (such as at least 85% for SEQ ID No. 7).

8. A polynucleotide according to claim 6 or 7 which comprises:

- (a) a sequence that encodes a polypeptide having synthase or transferase activity, which is:

- (1) the coding sequence of SEQ ID No. 1, 3, 5 or 7;
(2) a sequence which hybridises selectively to the complement of sequence defined in (1); or
(3) a sequence that is degenerate as a result of the genetic code with respect to a sequence defined in (1) or (2); or

(b) a sequence complementary to a polynucleotide defined in (a).

9. A polynucleotide according to any of claims 6 to 8 which is a DNA sequence.

10. A vector comprising one or more polynucleotide sequence(s) according to any one of claims 6 to 9.

11. A vector according to claim 10 which is an expression vector, such as where a DNA sequence according to claim 9 is operably linked to a regulatory sequence.

12. A host cell which comprises at least one polynucleotide according to any of claims 6 to 9, or has multiple copies of one or more of the polynucleotide(s).

13. A host cell which comprises, as a heterologous sequence, a polynucleotide according to any of claims 6 to 9.

14. A host cell, optionally prokaryotic, transformed with the DNA sequence, according to any of claim 6 to 9 or a vector of claim 10.

15. A process of producing or synthesizing a polypeptide according to any of claims 1 to 5 or vitamin B₁₂ or a precursor thereof, the process comprising culturing a host cell as defined in any of claims 12 to 14 under conditions that provide for

expression of the polypeptide or synthesis of vitamin B₁₂ or the precursor.

16. A composition comprising a polypeptide according to any one of claims 1 to 5.

17. A process for the preparation of an amine, the process comprising
5 contacting a substrate with an amide synthase from *Propionibacteria*, or a polypeptide comprising SEQ ID No. 2, or a variant or fragment thereof as defined in claim 3, or a host cell as defined in any of claims 12 to 14.

18. A process according to claim 17 wherein:

- 10 (a) the process is conducted in the presence of glutamine which is optionally converted to glutamate;
- (b) a carboxyl group is amidated to form a carboxyamide group;
- (c) the substrate is cobyrinic acid or cobyrinic acid c-diamide (Formula I or IA) and/or the product of the process is cobyrinic acid c-diamide or cobyrinic acid a,c-diamide (Formula IA or IB, respectively); and/or
- 15 (d) the process comprises amidating a substrate.

19. A process for the preparation of a phosphate-containing compound, the process comprising contacting a substrate with a phosphotransferase from *Propionibacterium*, a polypeptide comprising SEQ ID No.4 or a variant or fragment
20 thereof as defined in claim 3, or a host cell as defined in any of claims 12 to 14.

20. A process according to claim 19 wherein:

- (a) it is conducted in the presence of a nucleoside triphosphate, such as ATP;
- (b) the substrate comprises adenosine;
- 25 (c) the process comprises phosphorylation, optionally of a hydroxyl group; and
- (d) the substrate comprises adenosyl cobinamide (Formula II) and/or the product of the reaction is adenosyl cobinamide phosphate (Formula IIA).

30 21. A process for the preparation of a nucleotidyl-containing compound, the process

comprising contacting a substrate with a nucleotidyl transferase from

Propionibacterium, a

polypeptide comprising SEQ ID No. 4 or a variant or fragment thereof as defined in
claim 3, or a

5 host cell as defined in any of claims 12 to 14.

22. A process according to claim 21 wherein:

- (a) the process comprises guanidylating substrate;
- (b) the process comprises nucleotidylating a phosphate group;
- (c) the process is conducted in the presence of a nucleosyl triphosphate,
10 such as GTP; and/or
- (d) the substrate comprises adenosyl cobinamide phosphate (Formula IIA)
and/or the product of the reaction is adenosyl-GDP-cobamide
(Formula IIB).

23. A process for the preparation of an aryl-containing compound; the process
15 comprising

contacting a substrate with an aryl transferase from *Propionibacterium*, a
polypeptide comprising SEQ ID No. 6 or a variant or fragment thereof, as defined in
claim 3, or a host cell as defined in any of claims 12 to 14.

24. A process according to claim 23 wherein:

- 20 (a) the aryl moiety comprises an aromatic ring system of one or two rings,
optionally substituted with 1 to 4 C₁₋₈ alkyl groups, and with 0, 1 or 2
heteroatoms, optionally benzimidazole;
- (b) the product of the reaction has the aryl group bound to a transition
metal, such as cobalt, and to a carbon atom, optionally also to a ribose
25 group;
- (c) the process is conducted in the presence of a ribozole; and/or
- (d) the substrate comprises adenosyl-GDP-cobamide (Formula IIB) and/or
the product comprises adenosyl-5,6-dimethyl benzimidazolyl
cobamide (vitamin B₁₂, Formula IIC).

30 25. A process for the preparation of an adenosine-containing compound, the
process comprising contacting a substrate with an adenosyl transferase from

Propionibacterium, or a polypeptide comprising SEQ ID No. 8 or a variant or fragment thereof as defined in claim 3, or a host cell as defined in any of claims 12 to 14.

26. A process according to claim 24 wherein:

- 5 (a) the process comprises adenosylating a substrate, or the transfer of adenosine;
- (b) it involves the bonding of adenosine to a metal atom, optionally a transition series metal such as cobalt;
- (c) is conducted in the presence of a nucleosyl (tri) phosphate, such as
10 ATP; and/or
- (d) the substrate comprises cobyrinic acid a,c-diamide (Formula IB) and/or the product comprises adenosyl cobyrinic acid -a,c-diamide (Formula IC).

27. A process for producing vitamin B₁₂ or a precursor thereof, the process
15 comprising
culturing or fermenting a host cell according to any of claims 12 to 14 under
conditions such that
vitamin B₁₂ or the precursor precursor is produced or synthesised (such as by the
cell).

20 28. The use of a polypeptide according to any of claims 1 to 5, a polynucleotide according to
any of claims 6 to 9, a vector according to any of claims 10 or 11 or a host cell
according to any of
claims 12 to 11 in the manufacture or synthesis of vitamin B₁₂ or a precursor thereof.

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